

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

Claim 1 (currently amended): A separation matrix comprising a porous support comprising porous particles; and ligands coupled to the surfaces of said porous support, wherein the ligands provide at least one chemical gradient in the support, which said at least one chemical gradient is a continuous and smooth gradient, further wherein the chemical gradient(s) extend between the center and the exterior surface of each porous particle.

Claim 2 (cancelled)

Claim 3 (currently amended): The separation matrix of claim 1, wherein at least one chemical gradient is a ligand density gradient formed by a changing density of ligands on the support.

Claim 4 (previously presented): The separation matrix of claim 3, wherein two or more chemical gradients are present in the support and at least one gradient is a ligand density gradient.

Claim 5 (previously presented): The separation matrix of claim 3, wherein in the ligand density gradient(s), the ligand concentration increases towards the center of the support.

Claim 6 (previously presented): The separation matrix of claim 3, wherein in the ligand density gradient(s), the ligand concentration decreases towards the center of the support.

Claim 7 (currently amended): The separation matrix of claim 1, ~~which~~ wherein the matrix is a chromatography matrix comprised of a plurality of essentially spherical particles, wherein each particle presents one or more chemical gradient(s) perpendicular to the direction of the liquid flow applied in chromatography.

Claim 8 (currently amended): The separation matrix of claim 1, wherein at least one chemical gradient is the result of varying pKa values of functional groups of the ligands present on the support.

Claim 9 (currently amended): The separation matrix of claim 1, wherein at least one chemical gradient is the result of a varying net charge of the ligands present on the support.

Claim 10 (cancelled)

Claim 11 (currently amended): The separation matrix of claim 1, wherein the ligands present on the porous support provide at least two different functionalities.

Claim 12 (previously presented): The separation matrix of claim 11, wherein said functionalities are selected from the group consisting of cation exchange ligands, anion exchange ligands, hydrophobic interaction chromatography (HIC) ligands, reversed phase chromatography (RPC) ligands, immobilised metal chelating ligands (IMAC), thiophilic ligands, and affinity ligands.

Claim 13 (previously presented): The separation matrix of claim 11, wherein said at least two different functionalities are present on the same ligand.

Claim 14 (previously presented): The separation matrix of claim 11, wherein the ligands present zwitterionic functionalities.

Claim 15 (previously presented): The separation matrix of claim 11, wherein said at least two different functionalities are present on different ligand kinds, and each such ligand kind provides a separate chemical gradient within the support.

Claim 16 (withdrawn): A chromatography column packed with a separation matrix comprised of a porous support; and ligands coupled to the surfaces of said porous support, wherein the ligands provide at least one chemical gradient within the support.

Claim 17 (withdrawn): The chromatography column of claim 16, wherein the support comprises essentially spherical porous particles and the ligands provide at least one chemical gradient between the center and the exterior surface of each porous particle.

Claim 18 (withdrawn): The chromatography column of claim 16, wherein at least one gradient is a ligand density gradient formed by a changing density of ligands on the support.

Claim 19 (cancelled)

Claim 20 (withdrawn, currently amended): In a method of providing ~~a~~ the separation matrix ~~containing ligands coupled to the surfaces of a porous support of claim 1~~, the improvement comprises providing at least one ligand density gradient by solvent-controlled diffusion of at least one reagent into the porous support.

Claim 21 (withdrawn): The method of claim 20, wherein the solvent-controlled diffusion is obtained by contacting a first solvent comprising said reagent(s) with the

support, in the pores of which a second solvent is present, said first and second solvents presenting different solubilities.

Claim 22 (withdrawn): The method of claim 21, wherein the first solvent is aqueous and the second solvent is organic.

Claim 23 (withdrawn): The method of claim 21, wherein the first solvent is organic and the second solvent is aqueous.

Claim 24 (withdrawn): The method of claim 20, wherein the diffusion rate is controlled by adjusting one or more conditions selected from the group consisting of temperature; air flow; solvent properties; and concentration and/or nature of functionalities.

Claim 25 (withdrawn): The method of claim 24, wherein the diffusion of reagent(s) is assisted by providing an essentially continuous air flow through the reaction mixture during the reaction.

Claim 26 (withdrawn): The method of claim 20, wherein a ligand density gradient that decreases towards the center of the support is provided by diffusion-controlled addition to the porous support of a reagent that comprises at least one functionality.

Claim 27 (withdrawn): The method of claim 26, wherein either the reagent or groups present on the surface of the porous support have been activated before the reaction.

Claim 28 (withdrawn): The method of claim 20, wherein the support presents activated groups.

Claim 29 (withdrawn): The method of claim 28, wherein a ligand density gradient that increases towards the center of the support is provided by diffusion-controlled addition to the porous support of a first reagent, which comprises deactivating groups, to deactivate in a controlled fashion some of the surface groups of the support, and subsequent addition of another reagent, which comprises at least one functionality, to couple said at least one functionality to the surface groups that have not been deactivated.

Claim 30 (withdrawn): The method of claim 20, wherein the reagent comprises two different functionalities in a predetermined ratio to provide to different ligands in the separation matrix.

Claim 31 (withdrawn): The method of claim 20, wherein the porous support comprises essentially spherical particles.

Claim 32 (withdrawn, currently amended): A method of preparing ~~a separation matrix that comprises ligands coupled to the surfaces of a porous support~~ the separation matrix of claim 1, which method comprises the steps of:

- (a) providing activatable groups on the surface of a porous support;
- (b) activating said groups with an activation agent;
- (c) reacting groups activated according to step b) with a compound which comprises at least one functionality;

wherein control of the reactivity in step (c) results in at least one chemical gradient within the support.

Claim 33 (withdrawn): The method of claim 32, wherein the reactivity is controlled by the concentration of the compound that comprises the functionalities in step (c).

Claim 34 (withdrawn): The method of claim 32, wherein the activatable groups of step (a) are carbon-carbon double bonds.

Claim 35 (withdrawn): The method of claim 32, further comprising providing the activatable groups present at the surface of at least one porous particle in a step preceding step (a).

Claim 36 (withdrawn): The method of claim 35, wherein the step preceding step (a) comprises allylating hydroxyl groups present on the surface of a porous support.

Claim 37 (withdrawn): The method of claim 35, wherein steps (a)-(c) are replaced by a single step of reacting an activated ligand with the activatable groups present on the surface of the support.

Claim 38 (withdrawn): The method of claim 32, wherein the activation agent used in step (b) is a halogen.

Claim 39 (withdrawn): The method of claim 32, wherein at least one chemical gradient is a ligand density gradient.

Claim 40 (withdrawn): The method of claim 32, wherein at least one chemical gradient is a continuous and smooth gradient.

Claim 41 (withdrawn): The method of claim 32, wherein at least two different functionalities are provided in step (c).

Claim 42 (withdrawn): The method of claim 41, wherein said at least two different functionalities are provided by one compound.

Claim 43 (withdrawn): The matrix of claim 41, wherein said at least two different functionalities are provided by different compounds.

Claim 44 (withdrawn): The method of claim 32, wherein two or more chemical gradients are provided in the support, one of which is a ligand density gradient.

Claim 45 (withdrawn): The method of claim 32, wherein the support comprises porous particles and at least one gradient extends between the center and the exterior surface of each porous particle.

Claim 46 (withdrawn): A separation matrix prepared by the method of claim 20.

Claim 47 (withdrawn, currently amended): A process of liquid chromatography, comprising contacting a liquid including at least one target molecule with ~~a separation matrix, said separation matrix comprises ligands coupled to the surfaces of a porous support~~ the separation matrix of claim 1; and adsorbing the target molecule(s) to the matrix, wherein the ligands provide at least one chemical gradient within the support.

Claim 48 (withdrawn): The process of claim 47, wherein the liquid is applied in a flow direction which is perpendicular to at least one chemical gradient within the support.

Claim 49 (withdrawn): The process of claim 47, further comprising a step of eluting the adsorbed target molecule from the matrix by contacting the matrix with an eluent.

Claim 50 (withdrawn): The process of claim 47, wherein at least one chemical gradient is a ligand density gradient.

Claim 51 (withdrawn): The process of claim 47, wherein the support comprises essentially spherical porous particles and at least one chemical gradient extends between the center and the exterior surface of each porous particle.

Claim 52 (withdrawn): The process of claim 49, wherein the functional groups of the ligands are zwitterions and the elution is performed at a pH different from that during the adsorption.

Claim 53 (cancelled)

Claim 54 (withdrawn): The method of claim 45, wherein the porous particles are spherical particles.

Claim 55 (withdrawn): A separation matrix prepared by the method of claim 32.